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Oat protein concentrate as alternative ingredient for non-dairy yoghurt-type product

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Abstract:

BACKGROUND: During the industrial production of β-glucan, a protein-rich fraction remains as a by-product. Recovery of this protein as oat protein concentrate (OPC) results in a source of cereal protein for food and improves the overall economy of the process. In this study, a yoghurt-type product is developed by lactic acid fermentation of an OPC suspension after subjecting to heat treatment to assure starch gelatinization.

RESULTS: In detail, the process of yoghurt production involved an initial heating step to 90 °C, subsequently followed by 24 h fermentation with a starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* und *Streptococcus thermophilus*. The resulting yoghurt-type product was mildly sour (pH-value 4.2) with a certain amount of lactic acid (3.3±0.2 g kg^{-1}) and contained $4.9 \cdot 10^6$ cfu g^{-1} of lactobacillus after 24 h of fermentation. SEM revealed a porous network presumably built up from the gelatinized starch fraction containing aggregated structures in-between which were assumed to be aggregated oat proteins. Moreover, to a limited extent, proteolysis occurred during fermentation. Thus, some of the proteolytic enzymes being present in the yoghurt culture cleaved oat protein and released peptides. However, the effect on essential amino acids was small.

CONCLUSION: The results of this study provide a deeper knowledge into the role of starch and protein in fermented OPC yoghurts. The structure of fermented OPC verifies the applicability of oat protein as an alternative source for yoghurt-type products.

Keywords: oat protein concentrate, lactic acid fermentation, non-dairy yoghurt, plant proteins

INTRODUCTION

Plant proteins are gaining considerable interest as food ingredients with respect to the latest trends towards a healthy lifestyle, vegetarianism and veganism. Modern lifestyle-related chronic diseases such as obesity, insulin resistance and diabetes are the health challenges of the 21st century (1). As a result, the food industry aims to develop functional foods and functional food ingredients (for example probiotics and plant derived proteins), which exert positive effects on health and wellbeing of consumers. The most common probiotic foods are yoghurt or other fermented products containing various types of lactobacilli and bifidobacteria (2). As yoghurt-type products are already perceived as high protein food with high acceptability by consumers (3) they are a suitable system for the combined application of plant derived proteins and probiotics.

In the context of plant derived proteins, oat has a favourable composition and provides valuable nutrients for human consumption (4). Moreover, oat protein in the form of an oat protein concentrate is a sustainable raw material that arises as a protein-rich by-product from β-glucan production. Recovery of this protein as oat protein concentrate (OPC) results in a source of cereal protein for food and improves the overall economy of the process.

The globulins present in oat are insoluble under slightly acidic and neutral conditions (5,6). This limits the use in many food products, which usually show a pH between 3.0 and 7.0. The reason for the poor solubility is an unfolding of oat globulins, resulting in a transition from β-sheet to a random coil conformation (7,8) and formation of insoluble aggregates. As long as aggregation due to charge neutralization is known to be an essential step in the formation of a particle gel during yoghurt production (9), the aggregation behaviour of oat protein in an acidic environment should be advantageously applied to structure development of fermented OPC. With a denaturation temperature around 110 °C oat proteins are very heat-stable (10,11) and, thus, no protein denaturation occurs during the conventional heat-treatment. However, OPC contains a high amount of starch (approx. 300 g kg^{-1}) which gelatinizes at approx. 64 °C (10) and is supposed to take part in the structuring.

In general, cereals possess limitations in the amino acid profile, and in particular a reduced lysine content is the major disadvantage. Several methods can be used to improve the nutritional properties starting with genetic improvement, supplementation or fermentation (12). For wheat bran controlled fermentation was used to improve the amount of free amino acids thus improving the in vitro digestibility of the proteins (13). Besides this, fermentation benefits from an improved shelf life, texture, organoleptic properties and the possibility to produce new probiotic foods.

The aim of this work is (i) to gain a deeper insight into the characterization of the oat protein concentrate as a raw material for the use in semi-solid foods, (ii) the development of a process to gain a lactic fermented, non-dairy yoghurt type-product containing probiotic lactic acid bacteria and (iii) to analyse the impact of fermentation and growth of lactic acid bacteria on hydrolysis of protein. It is hypothesised, that gelatinization of starch prior to fermentation will help to create a starch network in which the protein is incorporated, thus pre-stabilizing the system. From a nutritional point of view, it is further hypothesised, that in analogy to other fermented product the amino-acid profile could be altered due to hydrolysis during fermentation.

MATERIALS AND METHODS

Oat protein concentrate (OPC) and oat protein isolate (OPI)

OPC was obtained according to the Kaukovirta-Norja et al. (14) patent for dry fractionation of oat grits with lipid removal by supercritical CO_2 extraction prior to milling and air classification. The resulting oat protein concentrate contained 35 ± 5 g kg^{-1} fat, 37 ± 0.3 g kg^{-1} ash, 421 ± 2 g kg^{-1} protein (Nx6.25), 447 ± 4 g kg^{-1} starch and 59 ± 4 g kg^{-1} total dietary fibre based on dry matter. OPI was produced from OPC by alkaline extraction according to the method described by Liu et al. (15) with slight modifications as described by Brückner-Gühmann et al. (16). OPI contained 870 g kg^{-1} protein and <10 g kg^{-1} starch.

Differential scanning calorimetry (DSC)

Thermal events corresponding to endothermic transitions which occur during heating of OPC were analysed using a DSC 8000 differential scanning calorimeter (Perkin Elmer, Inc., Waltham, MA, USA) calibrated with indium. The data obtained include enthalpy and specific temperatures characterizing heat-induced transition processes of the protein but also starch and other constituents. Approximately 5 mg of sample was weighed into a stainless-steel pan and deionized water was added at a ratio of 1:3. The sealed pan was left at room temperature overnight and then heated from 20-150 °C at a rate of 10 °C min^{-1} with 20 ml min^{-1} nitrogen flow rate. An empty pan was used as a reference. Peak temperature and enthalpy were calculated using Pyris Manager software (version 10.1, Perkin Elmer, Inc., Waltham, MA, USA). Analyses were performed in duplicate.

Fermentation

OPC (150 g kg^{-1}, i.e. 600 g OPC in 4 kg distilled water) was suspended under constant agitation in distilled water using a blender (Thermomix TM31, Vorwerk Deutschland Stiftung & Co. KG, Wuppertal, Germany) for 1 h. The suspension was continuously stirred and heated to 90 °C. Temperature was held for 30 min in the Thermomix and afterwards the sample was cooled down to 45 °C (fermentation temperature) in a water bath. 100 g kg^{-1} (w/v) lactose (400 g) (Sigma-Aldrich Chemie GmbH, Munich, Germany) and 2.8 g (0.2 units according to (16)) starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* und *Streptococcus thermophilus* (YC-X11 Yo-Flex, Chr. Hansen Holding A/S, Hoersholm, Denmark) were added to the system. Fermentation was performed for 24 h (16) at 45 °C. The OPC-yoghurt was cooled and stored at 4-6 °C in a refrigerator. Fermentations were done in triplicate. According to the specification of the yoghurt culture, the recommended incubation temperature is 35-45°C. In comparison to previous results in which cow milk was enriched with OPC and fermented at 40 °C (16), the fermentation of OPC alone required higher temperatures to induce microbiological action.

Microbiological analysis

Microbiological status of the fermented OPC was determined by analyzing colony forming units (cfu) after 24 h via cultivation on standard-I-agar (Nr. 1.07881.0500, Merck KGaA, Darmstadt, Germany). The concentration of lactobacillus of the fermented OPC after 24 h was determined by cultivation on MRS agar (Nr. CM1153, Oxoid Deutschland GmbH, Wesel, Germany) and china blue-lactose agar (Nr. 1.02348.0500, Merck KGaA, Darmstadt, Germany) at 37 °C under anaerobic conditions.

Determination of lactic acid and pH measurement

L-lactic acid concentration of the fermented OPC after 24 h was determined by a colorimetric assay for the determination of L-lactic acid in foodstuffs and other materials according to the instruction manual (R-biopharm AG, Darmstadt, Germany). The pH-value was measured every 2 hours during fermentation of OPC by WTW Microprozessor pH 95 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany).

Rheology

Rheological measurements of OPC fermentation were monitored up to 24 h in a rheometer (UDS 200, Anton Paar GmbH, Ostfildern, Germany) equipped with a concentric measuring cylinder (Z3 DIN). Oscillatory measurements started with time sweep mode at 45 °C up to 24 h (deformation: $\gamma = 10^{-3}$, frequency: 1 Hz) followed by a frequency sweep (deformation: $\gamma = 10^{-3}$, frequency: 0.01 to 10 Hz).

Scanning electron microscopy (SEM)

The microstructure of OPI model system (150 g kg^{-1}, pH 4, heat-treated for 30 min at 90 °C) and fermented OPC sample was analysed by SEM at the Center for Electron Microscopy (ZELMI), Technische Universität Berlin, Germany by the S-2700 scanning electron microscope (Hitachi, Tokyo, Japan). In preparation for SEM all samples were frozen in liquid nitrogen, freeze-dried and gold-sputtered in a sputter coater SCD 030 (Balzers, Wiesbaden-Nordenstadt, Germany); 1000-fold magnification.

High performance size exclusion chromatography (HPSEC)

HPSEC was performed to study changes in molecular mass of the proteins as a result of proteolysis by microorganisms. OPC samples were analysed in the course of fermentation after 0-2, 4, 8, and 24 h. Analysis was done at room temperature using an ÄKTAbasic™ 10 HPLC-system (Amersham Biosciences, Uppsala, Sweden) consisting of a separation unit (pump P-900, UV-monitor UV 900 operating at 280, 256 and 214 nm, UV flow cell (10 mm), injection valve INV 907, mixer M-925 and flow restrictor FR 904) and a personal computer running UNICORN™ control system (version 5.01 Amersham Biosciences, Uppsala, Sweden). The chromatographic column used was a Superdex 200 increase 10/300 GL (GE healthcare GmbH, Solingen, Germany). 0.2 M NaCl, 0.2 M sodium phosphate buffer, pH 7 was used as mobile phase with a flow rate of 0.75 ml min^{-1}. Prior to HPSEC all buffers were filtered and degassed with the mobile phase conditioner M 3522 (Bio Rad Laboratories GmbH, Munich, Germany).

An appropriate amount of OPC to make a 10 g kg^{-1} protein solution was dispersed in mobile phase, stirred for 90 min on a magnetic stirrer and left overnight in the refrigerator at 6 °C. Prior to chromatographic analysis the sample was allowed to equilibrate to room temperature. The samples were centrifuged at 10.000 g for 10 min at 10 °C (Sigma 3K12, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) to separate undissolved constituents.

Determination of free amino acids

Free amino acids were extracted from the sample using 6 M hydrochloric acid solution for 24 h at 110 °C. The macromolecules of the nitrogen compounds were precipitated with a solution

of sulfosalicylic acid and removed by filtration. Amino acids were converted to phenylthiocarbamyl (PTC) derivatives and determined by HPLC (Agilent 1260 Series, Agilent Technologies, Waldbronn, Germany) equipped with diode array detector (DAD)high performance liquid chromatography (HPLC-UV) with pre-column derivatisation. For tryptophan analysis, the sample was hydrolyzed in alkaline medium with NaOH with norleucine as internal standard and determined by HPLC with fluorimetric detection. Analysis was performed in triplicate. Analysis of variance (ANOVA) followed by Tukey's test was used to assess statistical differences between samples (Minitab®17 software, Minitab Ltd., UK). Differences were considered as significantly different at a value of $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of OPC

In the present study, OPC was fermented at a concentration of 150 g kg^{-1} with a protein concentration of approx. 55 g kg^{-1} and 6 g kg^{-1} starch. Prior to fermentation, the mixture was heated to 90 °C in order to completely gelatinize the starch fraction. DSC data describing heat-induced transitions of individual components present in OPC revealed three endothermic peaks at 64.2, 88.7 and 109.8 °C (Fig. 1). Fortunately, oat protein is very heat-stable with a denaturation temperature corresponding to the peak at 110 °C (OPI in Figure 1) (6,10,11). The amylose-lipid-complex transition (17) or denaturation of albumins (11) were supposed to occur at 89 °C. The peak at 64 °C corresponds to gelatinization of starch in line with previous findings (10). Thus, it can be supposed that the heating step involved in the production of the yoghurt-type product did not affect the protein structure but was enough to gelatinize the starch fraction. Moreover, the production of a stable, fermented product must include a gelatinization step of the starch fraction present in OPC.

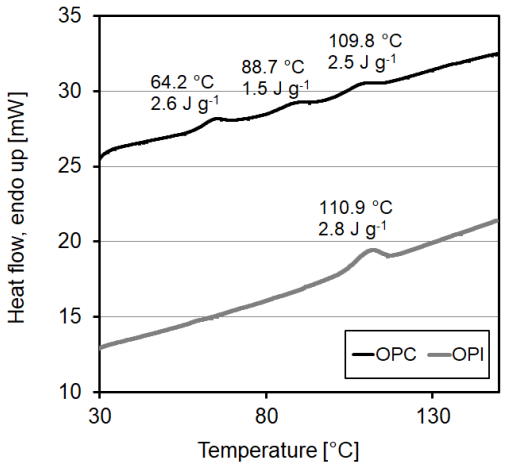

Figure 1. DSC thermogram of OPC and OPI

Lactic acid bacteria fermentation of OPC

The process of yoghurt production involved an initial heating step to 90 °C, subsequently followed by 24 h fermentation with a starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* und *Streptococcus thermophilus*. During fermentation, the initial pH of 6 decreased to 4.2 (Fig. 2a). In addition, after 24 h the product contained lactic acid at a concentration of 3.3±0.2 g kg^{-1}. The viable cell counts after 24 h fermentation ranged between

$3.9 \cdot 10^7$ to $4.5 \cdot 10^7$ cfu g^{-1} and the amount of lactobacillus after 24 h fermentation was $4.9 \cdot 10^6$ cfu g^{-1}. Thus, proving that a mildly sour, yoghurt-type product with a lactic fermented flavour was produced.

Viscoelastic properties as well as their frequency dependence were analysed by time and frequency sweeps in a rheometer. Figure 2a displays values of G' (storage modulus) and G'' (loss modulus) after 0, 2, 4, 6, 8 and 24 h of fermentation. A predominantly elastic behaviour (G' > G'') was found over time. This behavior was also verified by the course of the loss factor tan δ, which represents the relation of the elastic modulus, G', to the viscous modulus, G'', being minimal (tan δ < 1) over the tested range of frequencies (0.01 to 10 Hz) (Fig. 2b). According to the classification of Fernández Farrés et al. (18), fermented OPC can be described as soft fluid gel.

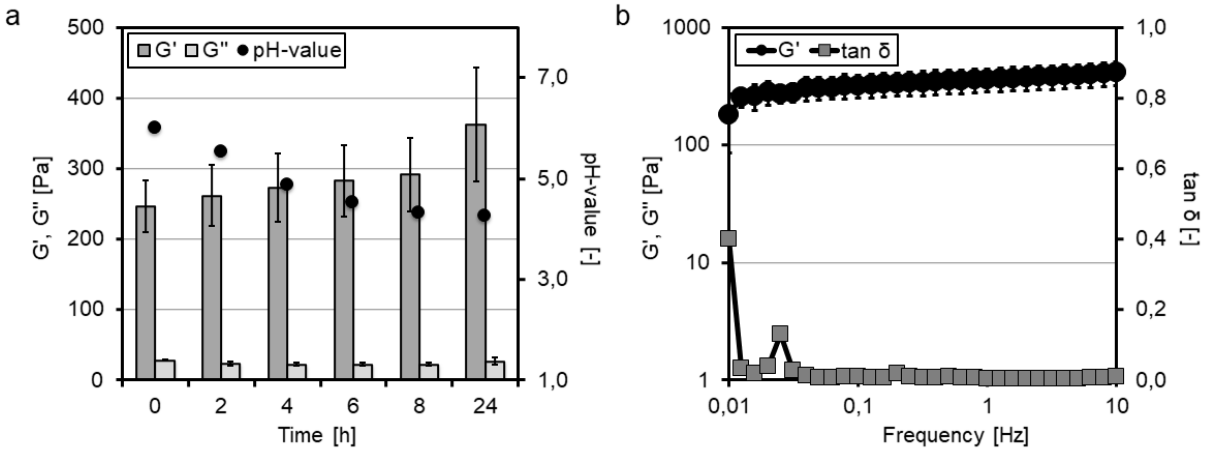

Figure 2. (a) Loss modulus G'' and storage G' modulus during fermentation of oat protein concentrate, (b) Frequency sweep of 24 h-fermented oat protein concentrate. Values are means of three independent replicates

In the course of fermentation, G' increased with decreasing pH pointing at a strengthening of structure due to the fermentation-caused decrease in pH (Fig. 2a). It can be hypothesised that upon heating above the gelatinization temperature of the oat starch and subsequent cooling, a starch gel was formed. Upon gelatinization of starch, starch granules absorb water and swell and amylose is leached, while upon cooling this amylose builds up a firm gel network with elastic properties (19) containing swollen starch granules as filler material (20). Assuming a low solubility of oat protein under acidic conditions (16) it is obvious that oat proteins start to aggregate and will be more or less physically incorporated into the starch gel network.

Analysis of microstructure

The microstructure of OPI model system (150 g kg^{-1}, pH 4, heat-treated for 30 min at 90 °C) and fermented OPC was analysed by scanning electron microscopy (Fig. 3). At pH 4, OPI model system showed no porous structure as a result of the low starch content but a high amount of aggregated structures presumably aggregated oat protein (Fig. 3a). The structure of fermented OPC was found to be a porous network built up from the gelatinized starch fraction containing aggregated structures in-between which were assumed to be aggregated oat proteins (Fig. 3b).

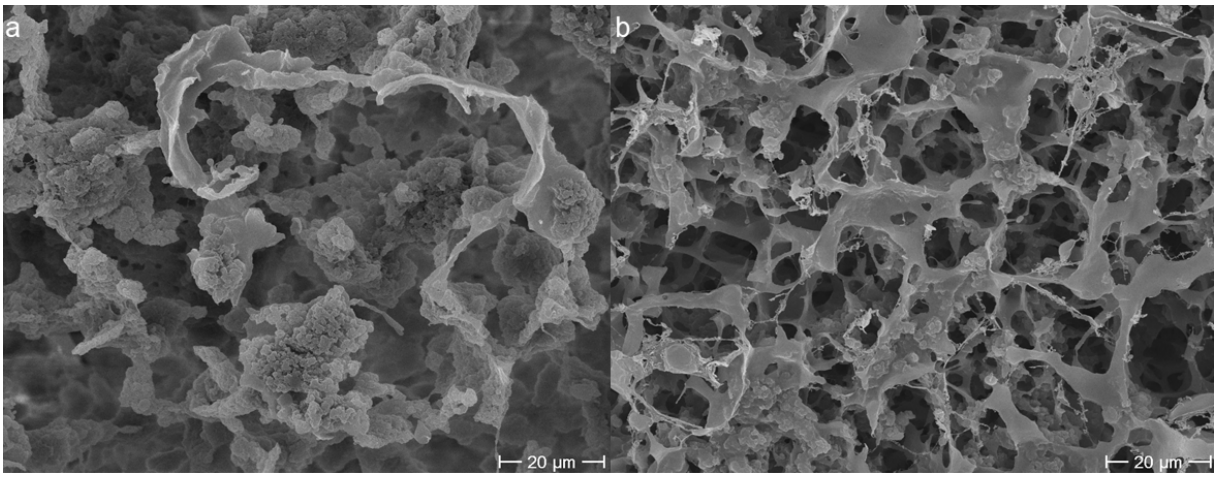

Figure 3. Scanning electron micrographs: (a) heated OPI suspension (150 g kg-1, 90°C) at pH 4; (b) 24 h fermented oat protein concentrate

Generally, when heated above the gelatinization temperature, starch granules in aqueous dispersion start to swell and amylose and amylopectin are solubilized. Upon cooling of concentrated suspensions, a gel of swollen, gelatinized starch granules dispersed in a network of amylose can be formed (21). It is hypothesized that the leached amylose builds up a rough macromolecular network (22) through non-covalent interactions (23), for example hydrogen bonding. It was proposed by Carnali and Zhou (1996) that swollen starch granules can act as filler material, increasing the rigidity of the amylose network towards small deformation at a given temperature and water content. In addition, deformability of the swollen granules was a factor influencing the rigidity of the starch paste. If the starch granules were less swollen due to a reduced amount of free water, the rigidity of the gel was increased (24). Carnali and Zhou (1996) described starch pastes as “naturally” reinforced polymeric materials. Rheological properties of starch pastes were determined by contributions and interactions of dispersed phase (filler material for example swollen starch granules) and dispersant (rough macromolecular network) (20,25). It is therefore likely, that the stability of a fermented yoghurt alternative based on OPC will be maintained by a starch gel rather than by a particle gel of aggregated oat protein.

Influence of fermentation on protein hydrolysis and amino acid profile

The relative size distribution obtained by HPSEC, which is the relevant integrated peak area as percentage of the overall chromatogram peak area is presented in Fig. 4a for four groups: soluble aggregates, hexamers, polypeptides and peptides (< 10 kDa). After 24 h of fermentation, only a small increase was detected for particles smaller than 10 kDa (from 69.4% at the beginning (0 to 2 h) to 72.56 after 24 h) and simultaneously, the peak representing the hexameric structure of the globulin decreased from 19.6% to 16% after 24 h of fermentation. Referring to the results of the amino acid profile (Fig. 4), fermentation of OPC resulted in a small increase of the tyrosine and lysine content which was not significantly different ($p > 0.05$) to unfermented OPC. Moreover, fermentation significantly decreased ($p < 0.05$) the cysteine content. The content of aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, valine, isoleucine and tryptophan also decreased after OPC fermentation, but did not differ significantly ($p > 0.05$) from that of OPC. On the other hand, fermentation did not significantly change ($p > 0.05$) the phenylalanine, methionine and leucine content.

It is very likely that, to a limited extent, proteolysis occurred during fermentation. Thus, some of the proteolytic enzymes being present in the yoghurt culture cleaved oat protein and released peptides. Unfortunately, the effect on essential amino acids was small.

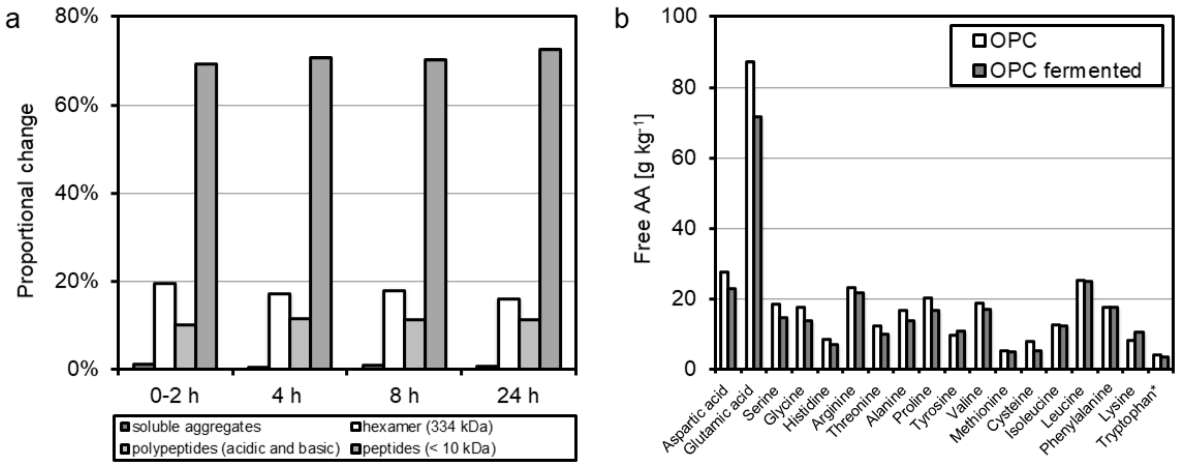

Figure 4. (a) Molecular weight distribution analysed by high-performance size exclusion at pH 7 at the beginning of fermentation and after 4, 6 and 24 h of fermentation (relative size distribution is the relevant integrated peak area as percentage of the overall chromatogram peak area), (b) amount of free amino acids of OPC, before and after fermentation. Values are means of two independent replicates

CONCLUSIONS

The present study clearly elaborates the potential of OPC as an alternative protein ingredient for non-dairy yoghurt-type products. Based on the findings, the following mechanism of structure development during fermentation of OPC may be formulated: at the beginning a thermal induced starch gel develops, in which the protein is incorporated. During fermentation, lactic acid is produced, which leads to an acidification of the product and increases gel properties. The results expand the knowledge on the role of starch and oat protein during structure formation in lactic acid fermented OPC suspensions. Fermentation by lactic acid bacteria resulted in a degradation of protein structure. Unfortunately, changes did not significantly increase the amount of essential amino acids. Future studies could focus on the improvement of this method. In addition, forthcoming research should include a controlled degradation of the starch in OPC by amylase to generate an inherent carbohydrate source for the growth of starter culture.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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